[CANCER RESEARCH 43, 5068–5071, November 1983]

Transport of Pyruvate in Mitochondria from Different Tumor Cells[1]

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ABSTRACT

A comparative study of the transport of pyruvate in mitochondria isolated from normal rat liver and from three tumors has been carried out. The K_m for net pyruvate uptake in mitochondria isolated from Ehrlich ascites tumor cells is practically equal to that measured in normal rat liver mitochondria while, on the other hand, it is higher in Morris hepatomas 44 and 3924A. The V_{max} of pyruvate uptake is depressed in all three types of tumor mitochondria as compared to that in the rat liver mitochondria, with the depression being higher in Morris hepatoma 3924A mitochondria. The lower activity of pyruvate translocator in mitochondria isolated from tumor cells as compared to that in rat liver mitochondria is also shown by depression of the rate of pyruvate-supported oxygen uptake. The results document a decreased activity of the pyruvate translocator in tumor mitochondria which seems to be correlated with the growth rate of the tumor cells.

INTRODUCTION

Rapidly growing tumor cells are characterized by a high rate of aerobic glycolysis (23). The molecular basis of this metabolic behavior is still largely unexplained, however (17, 24).

A crucial point in the regulation of aerobic glycolysis and of energy metabolism in general is represented by the transport of metabolites across the mitochondrial membrane from the cytosol to the matrix space of mitochondria. Previous work from this and other laboratories has shown the occurrence of alterations in the activity and kinetic parameters of anion translocators in tumor mitochondria with respect to normal tissues (4, 8, 14, 20, 21).

The pyruvate translocator mediates the transport of this substrate from the cytosol, where it is produced by glycolysis, to the mitochondrial matrix where it is aerobically degraded to CO_2 or carboxylated to oxaloacetate in the process of glucose resynthesis. The kinetics and substrate specificity of the pyruvate carrier have been investigated in detail (5, 6, 13, 15, 16). The properties of the pyruvate carrier have been shown to change in conjunction with different metabolic situations such as hormone treatment (7, 22) or pathological situations like diabetes (9). It has also been shown that pyruvate transport in mitochondria isolated from an Ehrlich hyperdiploid ascites tumor cell strain exhibits different kinetic parameters as compared to those in normal mitochondria (3).

As an extension of this work, we have carried out a comparative study on the transport of pyruvate in mitochondria isolated from 3 different tumors.

The results obtained indicate that the properties of the pyruvate translocator are affected differently in mitochondria isolated from tumor cells and that there exists a relationship between the decrease in the activity of the pyruvate transport system and the growth rate of tumors deriving from the same normal tissue.

MATERIALS AND METHODS

Ehrlich hyperdiploid ascites tumor cells were maintained by weekly i.p. transplantation in albino Swiss mice. Mitochondria were isolated as described previously (2). Morris hepatomas 44 and 3924A were transplanted into both hind legs of inbred rats of the Buffalo (i.m.) and ACI/T (s.c.) strains, respectively. The tumors, taken after 4 to 6 months (hepatoma 44) and 3 to 4 weeks (hepatoma 3924A), were carefully dissected free from adhering foreign tissue and necrotic and hemorrhagic areas. Mitochondria were isolated according to the methods of Schreiber *et al.* (18). Rat liver mitochondria were prepared as described previously (13). Protein was determined by the usual biuret method.

Pyruvate Transport. The initial rate of pyruvate uptake by rat liver mitochondria was measured by the centrifugation filtration technique as follows (see also Ref. 13). Mitochondria were preincubated at 20° in a reaction medium containing 150 mM sucrose, 30 mM Tris-HCl, 1 mM $MgCl_2$, 0.5 mM EDTA, 1 mM arsenite, oligomycin (10 μg/ml), rotenone (1.4 μg/ml), and antimycin (0.34 μg/ml). After 3 min preincubation, mitochondria were layered on the top of a second incubation layer at 4° and then spun down through this layer by rapid centrifugation. $HClO_4$ was immediately added to the mitochondrial pellet. The second incubation layer was of the same composition as the preincubation mixture with the addition of [^{14}C]pyruvate at the concentrations shown in the tables and charts. A discontinuous density gradient increasing towards the bottom of the centrifuge tube was made by addition of dextran to the second layer. The exposure time of mitochondria to the second incubation layer was estimated to be about 15 sec by measuring the oxidation of β-hydroxybutyrate to acetoacetate (see also Ref. 13). Pyruvate was measured in $HClO_4$ extracts of the mitochondrial pellet and in the supernatant. The substrate content of the matrix space was calculated by correcting the amount in the mitochondrial extract with that in the sucrose-permeable space plus adherent supernatant.

Measurements of the Rate of Oxygen Uptake. The rate of oxygen uptake was measured polarographically at 20° with a Clark electrode.

Determination of Transmembrane Electrochemical Proton Gradient. $\Delta\psi$ component of the aerobic proton electrochemical gradient was determined by the distribution of [^{3}H]methyltriphenylphosphonium bromide between supernatant and pellet, as indicated by Schuldiner and Kabach (19). $\Delta\psi$ was calculated by the Nernst equation. The concentration in the mitochondrial matrix was calculated by dividing the [^{3}H]-methyltriphenylphosphonium bromide uptake, corrected for sucrose-permeable space, by the matrix H_2O.

ΔpH was determined by the distribution of [^{14}C]dimethyloxazolidinedione across the mitochondrial membrane and calculated according to the method of Addanki *et al.* (1).

Mitochondria were incubated at room temperature in the incubation mixture described in the legend to Table 2 and bubbled with oxygen. Samples of the mitochondrial suspension were then centrifuged for 1 min at 20,000 × *g*. Aliquots of the $HClO_4$ extract of the pellet and supernatant were counted for radioactivity.

The matrix volume in respiring mitochondria was determined in sepa-

[1] This work has been supported by Grants 78.02854.96, 80.01606.96, and 80.01659.96 from Progetto Finalizzato Controllo della Crescita Neoplastica, Consiglio Nazionale delle Ricerche, Rome, Italy.

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Received February 3, 1982; accepted July 27, 1983.

rate samples with the use of ^3H_2O (10 μCi/ml) to measure the total pellet water and [^{14}C]sucrose (10 μCi/ml) to measure the extra matrix water. The matrix volume was calculated from the difference between ^3H_2O and [^{14}C]sucrose-permeable H_2O.

Chemicals. ^3H_2O, [^{3}H]methyltriphenylphosphonium bromide, and [^{14}C]dimethyloxazolidinedione were obtained from New England Nuclear, Dreieich, West Germany. [^{14}C]Sucrose and [*U*-^{14}C]pyruvate were obtained from the Radiochemical Centre, Amersham, England. Labeled pyruvate was treated as follows. The solid was dissolved in water, divided into 5-μCi samples, freeze-dried, and stored in sealed tubes at −20°. All chemicals were purchased from Sigma Chemical Co., St. Louis, Mo., or Boehringer Mannheim Co., Mannheim, West Germany. The chemicals used were of the highest-purity standards commercially available.

RESULTS

Chart 1 illustrates the kinetics of pyruvate uptake in mitochondria isolated from normal rat liver, Ehrlich ascites tumor cells, Morris hepatoma 44, and Morris hepatoma 3924A. Double reciprocal plots of net pyruvate uptake followed saturation kinetics in all 4 types of mitochondria. The affinity of the carrier for pyruvate in ascites tumor cell mitochondria is the same as that in rat liver mitochondria. The K_m for pyruvate is somewhat increased in mitochondria isolated from Morris hepatomas 44 and 3924A.

The maximal velocity of pyruvate uptake is significantly depressed in all 3 types of tumor mitochondria as compared to that in rat liver mitochondria. The degree of this depression is almost the same for ascites tumor cell mitochondria and Morris hepatoma 44 mitochondria as compared to that in rat liver mitochondria, the V_{max} values being 11.9 ± 0.6 (S.E.), 11.6 ± 0.8, and 19.8 ± 0.5 nmol/min/mg of protein, respectively; it is more pronounced in mitochondria from Morris hepatoma 3924A, in which the V_{max} drops to 4.7 ± 0.5 nmol/min/mg of protein.

Assessment of the capacity of mitochondria from tumor cells to take up and utilize pyruvate was also made by following pyruvate-stimulated oxygen uptake. Chart 2 shows that pyruvate-stimulated oxygen uptake (at 0.05 to 2.0 mM), corrected for endogenous respiration, was considerably lower in mitochondria from Ehrlich ascites cells and Morris hepatoma 3924A than that in rat liver mitochondria. On the contrary, no significant differences in the rate of oxygen uptake between normal and tumor cell mitochondria could be noted when succinate was used as respiratory substrate. The statistical analysis of pyruvate-supported respiratory rates in mitochondria from rat liver and tumor cells is presented in Table 1.

It has been established that the uptake of pyruvate by mitochondria is driven by transmembrane pH gradient (13). Observations (12) from our laboratories have revealed a decreased

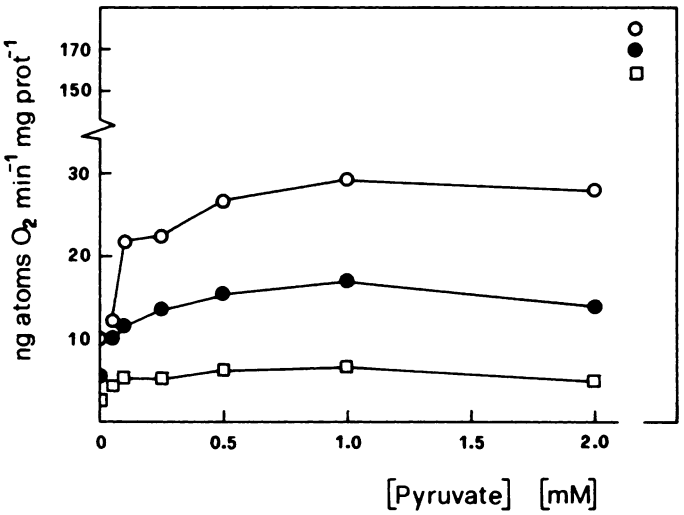

Chart 2. Pyruvate-supported oxygen uptake in mitochondria isolated from normal rat liver, Ehrlich ascites tumor cells, and Morris hepatoma 3924A. Mitochondria [3 mg protein (*prot.*) per ml] were incubated in a reaction medium containing 75 mM sucrose, 30 mM Tris-HCl, 50 mM KCl, 1 mM $MgCl_2$, 0.5 mM EDTA, 1 mM potassium phosphate, and 0.3 μM carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone. Final pH, 7.0. Temperature, 20°. Pyruvate-supported respiratory rates were corrected for endogenous respiration. The values reported are the means for 3 experiments. *Points* at *top right*, respiratory rates measured with 5.0 mM succinate as respiratory substrates.

Points on *left ordinate*, endogenous respiratory rates measured after 5 min preincubation of mitochondria just before pyruvate addition. ○, rat liver; ●, Ehrlich ascites tumor cells; □, Morris hepatoma 3924A.

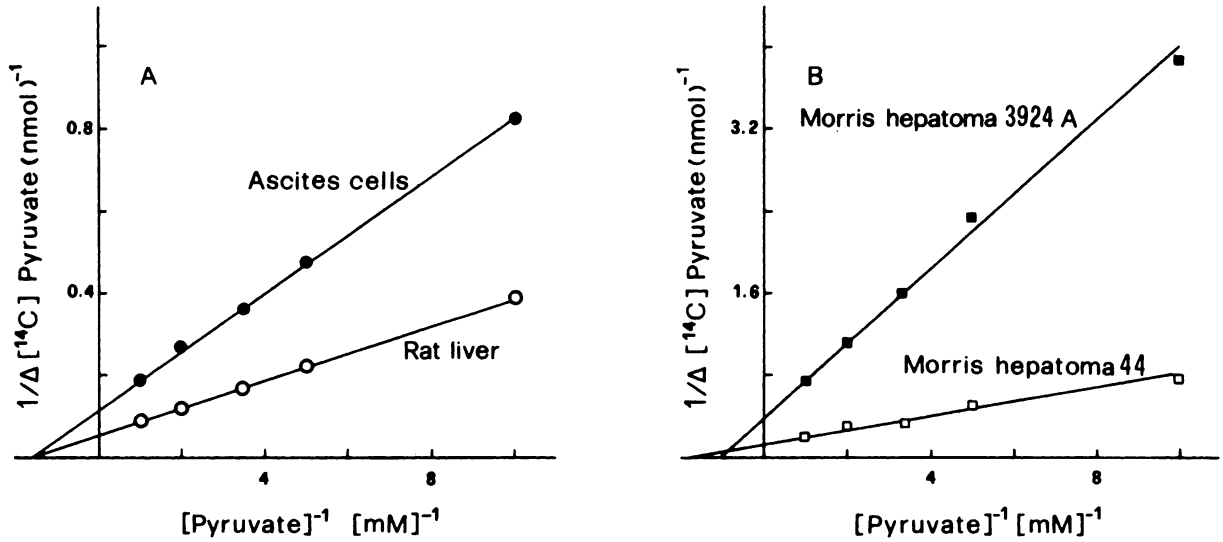

Chart 1. Double reciprocal plots of pyruvate uptake by mitochondria from rat liver, Ehrlich ascites tumor cells, Morris hepatoma 44, and Morris hepatoma 3924A. Mitochondria (2.5 mg protein per ml) were preincubated in the sucrose medium described in "Materials and Methods." Final pH, 7.0. Temperature, 20°. After 3 min, mitochondria were centrifuged through a second layer at 4° containing the same components as the preincubation medium plus labeled pyruvate at the concentrations indicated in the chart.

Table 1

Statistical analysis of the kinetic parameters of pyruvate uptake and pyruvate-supported respiratory rates in mitochondria isolated from normal rat liver, Ehrlich ascites tumor cells, Morris hepatoma 44, and Morris hepatoma 3924A

The kinetic parameters of pyruvate uptake were determined as described in the legend to Chart 1. The rate for oxygen uptake supported by 0.5 mM pyruvate was measured as described in the legend to Chart 2. For other experimental details, see "Materials and Methods."

Mitochondria	Pyruvate uptake		Rate of oxygen uptake supported by pyruvate (nanoatoms/min/mg protein)
	K_m (mM)	V_{max} (nmol/min/mg protein)	
Rat liver	0.64 ± 0.01[a] (6)[b]	19.8 ± 0.5[a] (6)[b]	23.5 ± 1.8[c] (8)[d]
Ehrlich ascites	0.63 ± 0.02 (5)	11.9 ± 0.6 (5)	14.5 ± 2.2 (8)
Morris hepatoma 44	0.74 ± 0.03 (4)	11.6 ± 0.8 (4)	13.3 ± 2.5 (3)
Morris hepatoma 3924A	1.1 ± 0.07 (4)	4.7 ± 0.5 (4)	7.5 ± 2.7 (4)

[a] Mean ± S.E.
[b] Numbers in parentheses, number of experiments.
[c] Mean ± S.E. of values corrected for endogenous respiration.
[d] Numbers in parentheses, number of determinations.

Table 2

Aerobic transmembrane $\Delta\tilde{\mu}H^+$ in rat liver and tumor cell mitochondria

Mitochondria (1.5 mg protein per ml) were suspended at room temperature in a medium containing 100 mM sucrose, 20 mM KCl, 20 mM *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid, 0.5 mM EDTA, *N*-ethylmaleimide (30 nmol/mg protein), rotenone (1 μg/mg protein), oligomycin (2 μg/mg protein), 10 μM [^{3}H]methyltriphenylphosphonium bromide (2 μCi/ml), or 20 μM [^{14}C]dimethyloxazolidinedione (2 μCi/ml). Final volume, 1 ml. pH was adjusted to 7 with KOH.

Mitochondria	$\Delta\psi$ (mV)	−59 ΔpH (mV)	$\Delta\tilde{\mu}H^+$ (mV)
Rat liver	129	73	202
Ehrlich ascites cells	120	76	196
Morris hepatoma 44	126	68	194
Morris hepatoma 3924A	105	61	166

capacity of tumor mitochondria to conserve aerobic ΔpH, when the organelles are suspended in a Na^+-containing medium.

The results reported in Table 2 show that, when mitochondria were suspended in a K^+ medium at pH 7.0, such as that used for following pyruvate uptake, there was no appreciable change in transmembrane ΔpH and $\Delta\psi$ generated by succinate respiration in ascites tumor cells and Morris hepatoma 44 mitochondria as compared to that in rat liver mitochondria. A small decrease of both the ΔpH and $\Delta\psi$ could be noted in mitochondria isolated from Morris hepatoma 3924A.

DISCUSSION

The results presented in this paper show that pyruvate transport in mitochondria isolated from 3 different types of tumor cells is depressed with respect to that in rat liver mitochondria. The V_{max} of the pyruvate carrier in mitochondria isolated from ascites tumor cells and Morris hepatoma 44 amounts to 60%, and that from Morris hepatoma 3924A mitochondria amounts to 25% of that exhibited by rat liver mitochondria. On the other hand, the affinity of pyruvate for its carrier is practically unaffected in mitochondria from ascites tumor cells and Morris hepatoma 44 as compared to that in rat liver mitochondria. Only mitochondria from Morris hepatoma 3924A show a significant decrease in affinity.

Data have been reported suggesting that pyruvate transport may limit mitochondrial pyruvate metabolism (6, 15). Slower pyruvate penetration into mitochondria may be responsible for the lower rate of pyruvate-supported oxygen consumption in tumor cell mitochondria. The data of Chart 2 and Table 1 demonstrate a direct relationship between the degree of inhibition of pyruvate transport and that of pyruvate oxidation in the 3 types of tumor mitochondria as compared to that in the rat liver mitochondria.

Changes in the activity of pyruvate transport in mitochondria could depend either on factors affecting this process directly or on factors causing collapse of the transmembrane pH gradient. Measurements of aerobic $\Delta\mu H^+$ under conditions close to those used to follow the kinetics of pyruvate uptake in a K^+ medium show that under these conditions there is no significant decrease of ΔpH (see Table 2). Thus, the depression of pyruvate uptake by tumor cell mitochondria, shown in the present work, can be ascribed directly to a lower activity of the transport system.

This could reflect a lower content of carrier molecules in the mitochondrial membrane. However, the possibility that lower activity of pyruvate transport in tumor cell mitochondria is due to changes in the level of endogenous substances which affect the translocater should be taken into account.

Morris hepatomas 44 and 3924A are characterized by different growth rates and states of differentiation, the former being a well-differentiated, slowly growing tumor, the second being a poorly differentiated, rapidly growing tumor. The properties of the pyruvate translocator are more affected in mitochondria from Morris hepatoma 3924A than they are in those from Morris hepatoma 44. This would suggest the existence of a relationship between the degree of alteration in the activity of the mitochondrial pyruvate-transporting system and the degree of the growth rate and state of tumor differentiation deriving from the same normal tissue.

Pyruvate represents one of the most important substrates in intermediary metabolism. Alterations in the properties of the mitochondrial pyruvate-transporting system, in conjunction with modifications of enzymatic activities involved in pyruvate metabolism, such as pyruvate dehydrogenase (10), may contribute in addition to other factors to the reported derangements of energy metabolism in cancer cells (*e.g.*, enhanced aerobic glycolysis, enhanced contribution of oxidation of fatty acids and of ketone bodies to energy supply, etc.).

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